ЕФЕКТ ОТ ТОРЕНЕТО ВЪРХУ НЯКОИ ФИЗИОЛОГИЧНИ ПАРАМЕТРИ ПРИ ЛЮТИВ ПИПЕР (CAPSICUM ANNUUM L.) EFFECT OF FERTILIZATION ON SOME PHYSIOLOGICAL PARAMETERS OF HOT PEPPER (CAPSICUM ANNUUM L.)

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Резюме

Изследвано е влиянието на два минерални тора – Substral и Agrolex, върху физиологичните параметри на лютив пипер (*Capsicum annuum* L.) при полски условия. В изследването са включени три сорта: "Вулкан", "Тракийска шипка" и "Чорбаджийски". Доказано е, че торенето с Agricol оказва по-добър ефект върху съдържанието на свободен пролин, пластидните пигменти, листния газообмен и водния баланс.

Abstract

The effect of fertilization on the physiological parameters (content of free proline, content of plastid pigments, intensity of CO₂ assimilation and transpiration and water balance) in three hot pepper (*Capsicum annuum* L.) cvs. *Wulkan, Trakijska Shipka, Chorbadgijski* under field conditions was studied. Two mineral fertilizers were used in the experiment: *Substral* and *Agrolex*. The results showed that the treatment with *Agrecol* produced a better result in the hot pepper plants than *Sunstral*.

Ключови думи: Capsicum annuum L., торене, пластидни пигменти, газообмен, пролин. Key words: Capsicum annuum L., fertilization, plastid pigments, gas exchange, proline.

INTRODUCTION

Pepper (*Capsicum annuum* L.) ranks among the most important vegetable crops worldwide. It is regarded as a vegetable and a condiment (Bosland & Votava, 1999; Mezghani et al., 2007).

Fertilizer treatments, are associated with the fertilization of substrates and are targeted for optimal plant nutrition (Golcz et al., 2004), that contributes to a high value crop quality and quantity, having regard to environmental requirements and ecological aspects (Golcz et al., 2009).

Proper growth and development of plants is strongly correlated with a balanced mineral feeding (Sady and Smoleń, 2007). Nutrients such as nitrogen, phosphorus and potassium have a significant influence on plant growth and development (Choi et al., 2008). They also determine the life processes occur in plants (Zamin Shaheed Siddiqui, 2007).

The aim of this study was to determine the effect of fertilization on physiological indices (content of free proline, content of plastid pigments, intensity of CO_2 assimilation and transpiration and water balance) in plants

of hot pepper (*Capsicum annuum* L.) cv. 'Wulkan', 'Trakijska Shipka', 'Chorbadgijski'.

MATERIALS AND METHODS

The experiment with hot pepper (*Capsicum annuum* L.): 'Wulkan', 'Trakijska Shipka', Chorbadgijski' was carried out in 2011 in the Vegetable Research Station in Dołuje near Szczecin.

Experiment was conducted in the random block design with three replications. Seedlings of pepper were produced in the greenhouse. Seeds were sown in the third decade of March to the multiplies, after dry seed coating them with fungicide T (75% thiram - dithiocarbamate). Three weeks after sowing, seedlings were moved to pots. Taken off a liquid foliar fertilizer Florowit (N - 3,0; $P_2O_5 - 0,69$; $K_2O - 2.3$; MgO - 0,1; CaO - 0,75; S - 0,2) at a concentration of 0.3%.

The field before planting plants was fertilized (taking into consideration the analysis of soil). Nutrients complemented to the level of 120 N, 80 P, 250 K, 70 Mg and Ca mg.dm³ soil. Phosphorus and potassium and half

of the nitrogen applied in spring. Rest dose of nitrogen applied: first 3 weeks after planting plants, and another twice at intervals of 1-3 weeks.

Seedlings were planted in early June on experimental plots of size 3.2 m^2 ($2.0 \times 1.6 \text{ m}$). After three weeks from planting crops on the field, soil-applied fertilizers: Agrolex (N - 10% (NO₃ - 3,9%, NH₄ - 6,1%); $8\% - P_2O_5$; $K_2O - 22\%$) on one item), at a dose of 0,5 g per 1 L of water and Substral (N - 9% (NO₃ - 6.6%, 2.4% - NH₄), $P_2O_5 - 9\%$, $K_2O - 27\%$, MgO 3%, B - 0,01%, Cu - 0,01%, Fe - 0,06%, - Mn 0,04%, Mo - 0,001%, Zn - 0.01%) at a dose of 30 g per m². Spraying was made on one object each cultivar in each replicate. Three times in the two-week intervals were made identical spraying the same means.

Measurements of the studied traits were performed three times at intervals of 9-day intervals. The first measurement was made a month after the last main fertilization (Agrolex, Substral).

Plastid pigments (chlorophyll 'a', chlorophyll 'b', carotenoids) were determined by the method of Lichtenthaler and Wellburn (1983). The fresh leaf samples (approximately 0,05 g) were triturated in a mortar with 10 ml of 80% acetone. After receiving the sample, extract was centrifuged for 10 minutes at 1500 revolutions . min⁻¹. Then the clear solution was decanted and made up 80% acetone to 10 ml. The absorbance of the prepared supernatants was determined in a spectrophotometer MacelMini at wavelengths: 440, 645 and 663 nm. Plastid pigments content in leaves of pepper was calculated by Arnon et al. (1956).

In order to determine the water balance indicators (RWC, WSD), leaves (fresh plant material) were weighed on an analytical balance with a precision of 0.001 g Then they were placed for 24 hours in a glass vessel filled with distilled water, then removed and dried with tissue paper. Leaves were weighed again to determine the weight of leaves at full saturation with water. The next stage of the plant material was, drying in an oven to constant weight (at 80°C for 24 hours), and re-weighing. The obtained data were converted by patterns of two indicators (Bandurska, 1991; Kopcewicz, Lewak, 2002).

Measurements of CO₂ assimilation (μ mol.m⁻².s⁻¹), leaf transpiration (mmol.m⁻².s⁻¹), stomatal conductance (mol.m⁻².s⁻¹) and CO₂ content in the intercellular expanses (μ mol CO₂.mol⁻¹) were made using TPS-2 gas analyzer, produced by PP Systems company, working in an open system in which air was passing through the apparatus in a continuous manner. Apparatus was equipped with a leaf chamber with a lamp mounted on the light intensity 2053 μ mol.m⁻².s⁻¹.

For the determination of free proline content of the plant material was homogenized in 10ml of 3% aqueous solution of sulfosalicylic acid. The resulting homogenate was filtered through Whatman filter paper (No. 2) and made up to a volume of 10ml. Proline content was determined by Bates et al. (1973). For the determination 2 ml of the filtrate was collected and added 2 ml of glacial acetic acid and 2 ml reagent prepared from 1,25 g of ninhydrin dissolved in 30 ml of glacial acetic acid and supplemented with 6 mol (H_3PO_4) dm⁻³ for 50ml. The samples were placed for 1 hour in a boiling water bath. After cooling, they were shaken with 4 ml of toluene and allowed to phase separation. The upper phase was gently taken for the determination. Extinction was measured on a spectrophotometer MarcelMini at a wavelength of 520 to toluene.

The results were developed in the program "Statistica 9.1" produced by Statsoft. For statistical calculations univariate analysis of variance was used. The significance of differences between the variants of experiments were determined by Duncan test at significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The highest concentration of chlorophyll "a" was found in cv. 'Chorbadgijski' treated by Agrecol. The lowest chlorophyll "a" was recorded in plants of 'Trakijska Shipka' (control). The plants of 'Trakijska Shipka' treated by Agrecol, showed a significantly higher content of chlorophyll "b" in the leaves compared to the control of 'Trakijska Shipka' where it was found significantly lower contents of this dye. Total chlorophyll content in fresh hot pepper leaf weight showed differences depending on the applied fertilizer. The highest average content of chlorophyll a + b was characterized in cv. 'Trakijska Shipka' (control), and the lowest of that indicator were in cv. 'Wulkan' (control) -(Table 1).

The highest values of relative water content in the leaves (RWC) was observed in plants of cv. 'Trakijska Shipka' (which represented 88,40% of control), while the lowest were characterized by cv. 'Wulkan'' (66,68% of Agrecol). Noted a significant increase of water in cells of cv. 'Chorbadgijski (treatment with Agrecol and Substral). The lowest value of WSD indicator was recorded in the cv. 'Trakijska Shipka' (11,60% of control), while the highest value was characterized by cv. 'Wulkan' (31,34% of control) - (Fig.1).

Significant influence of fertilization applied on the intensity of gas exchange processes, as well as stomatal conductance and CO_2 concentration in the intercellular expanses was found. The lowest values of the intensity of the process of assimilation were noted in the plants of cv. 'Wulkan' and 'Trakijska Shipka' (both 0,27 µmol $CO_2 \cdot m^2 \cdot s^{-1}$ of Substral), while the highest in the absence of cv. 'Trakijska Shipka' (control) and 'Chorbadgijski' (Agrecol) (Table 2.). Plants that were growing in the fertilization variant of Substral of cv. 'Wulkan' were characterized by significantly lowest transpiration intensity (0,25 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$). Significantly the highest value of stomatal conductance was

 Таблица 1. Влияние на торенето върху съдържанието на пластидни пигменти (mg g⁻¹ св. тегло) на растения от три сорта пипер

 Table 1. Influence of fertilization on the content of plastid pigments (mg g⁻¹ fresh weight) of plants of three cultivars pepper

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Сортове - торове Cultivar – fertilizer	Съдържание на хлорофил "a" Content of chlorophyll "a"		Съдържание на хлорофил "в" Content of chlorophyll "b"		Съдържание на каротеноиди Content of carotenoids		Хлорофил "a"/ хлорофил "в" Chlorophyll "a"/
	(mg · g⁻ˈ fr.w.)	h.g.	(mg · g⁻' fr.w.)	h.g.	(mg · g⁻' fr.w.)	h.g.	chlorophyll "b"
Wulkan - Control	1,463	Α	0,650	AB	0,936	AB	2,25
Trakijska Shipka - Control	1,303	А	0,534	А	0,836	А	2,44
Chorbadgijski - Control	1,519	AB	0,649	AB	0,967	AB	2,34
Wulkan - Agrecol	1,532	AB	0,675	AB	0,965	AB	2,27
Trakijska Shipka - Agrecol	1,642	В	0,711	В	0,994	AB	2,31
Chorbadgijski - Agrecol	1,676	В	0,693	AB	0,961	AB	2,42
Wulkan - Substral	1,414	А	0,587	Α	0,942	AB	2,41
Trakijska Shipka - Substral	1,469	AB	0,617	AB	0,976	AB	2,38
Chorbadgijski - Substral	1,619	AB	0,705	В	1,146	В	2,30



Фиг.1. Влияние на торенето върху водния баланс (%); Wul - Вулкан, TS - Тракийска шипка, Czarb - Чорбаджийски Fig.1. Influence of fertilization on the water balance indicators (%); Wul - Wulkan, TS - Trakijska Shipka, Czarb - 'Chorbadgijski reported in plants of cv. Chorbadgijski treated by Agrecol (32,67 mol·m⁻²·s⁻¹), while the highest content of CO_2 in the intercellular expanses was found in plants grown in control of cv. "Chorbadgijski" (Table 2).

The significant differences in the content of free proline in the plants, was observed between cv. 'Trakijska Shipka' treated with Agrecol (30,94 μ mol.g⁻¹ f.m.), and cv. Chorbadgijski treated with Substral (7,02 μ mol.g⁻¹ f.m.) - (Fig. 2).

Studies of Choi et al. (2008) have shown that a balanced fertilization is essential for successful growth and development of hot pepper. Deficiency of one of the major elements in plant nutrients (nitrogen, phosphorus, potassium) can lead to disorders of physiological parameters (Choi et al., 2000; Lindsay, 1979; Marschner, 1995).

Piskornik (1999) and Myczkowski (1976) based on its research indicate that the correct ratio of chlorophyll

Габлица 2. Влияние на торенето върху листния газов обмен на растения от три сорт	а пипер
Table 2. Influence of fertilization on the leaf gas exchange of plants of three cultivars p	epper

Сорт Cultivar	Вариант Variant	Асимилация на CO₂ Assimilation CO₂		Транспирация Transpiration		Устична проводимост Stomatal conductance		Концентрация на CO ₂ в междуклетъчното пространство Concentration of CO ₂ in intercellular expanses	
		(µmol CO₂ m⁻² s⁻¹)	h.g.	(mmol H ₂ O ·m ⁻² ·s ⁻¹)	h.g.	(mol ⁻² ·s ⁻¹)	h.g.	(µmol CO₂ · mol⁻¹)	h.g.
Wulkan	Control	0,33	AB	0,27	Α	24,67	AB	55,00	В
Trakijska Shipka	Control	0,43	В	0,31	AB	27,67	AB	57,00	В
Chorbadgijski	Control	0,40	В	0,40	В	27,00	AB	81,67	С
Wulkan	Agrecol	0,37	AB	0,33	AB	26,67	AB	41,00	Α
Trakijska Shipka	Agrecol	0,33	AB	0,36	AB	29,00	AB	48,67	AB
Chorbadgijski	Agrecol	0,43	В	0,33	AB	32,67	В	48,00	AB
Wulkan	Substral	0,27	Α	0,25	Α	19,00	AB	57,67	В
Trakijska Shipka	Substral	0,27	A	0,27	А	20,00	AB	49,67	AB
Chorbadgijski	Substral	0,40	В	0,23	Α	15,67	А	55,67	В



Фиг.2. Влияние на торенето върху съдържанието на свободен пролин (µmol.g⁻¹ f.w.); Wul - Вулкан, TS - Тракийска шипка, Czarb - Чорбаджийски Fig.2. Influence of fertilization on the content of free proline (µmol.g⁻¹ f.w.); Wul - Wulkan, TS - Trakijska Shipka, Czarb – 'Chorbadgijski

a and b, in plants grown in our climate should be from 2.3 to 5.5, while the carotenoids content should be about 3.5 times less than the total chlorophyll content. In the conducted experiment results did not differ from the literature data, which indicates the correctness of the synthesis of dyes.

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Studies conducted by Cramer et al. (2009) have shown that there is a correlation between the collection of available nitrogen by plants, and water content in tissues. Deficiency of this element in the soil reduces the saturation of water in the leaf. The influence of nitrogen on the water balance indicators has also been noted in case of own research. The plants which were treated by fertilizer with reduced nitrogen (Substral) content showed decrease of water saturation. The results show that treatment with Agrecol has better result of hot pepper plants than Sunstral.

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